Studies on the Constituents of Edible and Medicinal Plants. III. Effects of Seven Limonoids on the Sleeping Time Induced in Mice by Anesthetics

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Effects of seven limonoids, obakunone (1), 7α-obakunol (2), 7β-obakunol (3), limonin (4), 7α-limonol (5), 7β-limonol (6) and nomilin (7), on the sleeping time induced in mice by anesthetics were assayed.

All the limonoids, except for 2, shortened the sleeping time induced by α-chloralose and urethane. 7 gave the highest reduction rate of sleeping time, and the order of the reduction rate of sleeping time was as follows; 7>1 and 3>4, 5 and 6.

As the chemical structures of these compounds are similar to each other, the relationship between the structure and the effects of limonoids on sleeping time was discussed.

Keywords limonoid; sleeping time; anesthetic; α-chloralose; urethane; nomilin; obakunone; limonin; obakunol; limonol

Limonoids are modified triterpenes present in the plant order Rutales. There are many bitter limonoids in citrus fruits, and they are largely responsible for the delayed development of bitterness in citrus juices.[1] This delayed bitterness is a serious economic problem in the citrus industry. On the other hand, recently some noteworthy reports on the biological activities of limonoids have been reported,[2] and in the previous paper,[3] we reported that obakunone (**1**) and limonin (**4**), which are the typical bitter limonoids, shortened the sleeping time induced in mice by α-chloralose and urethane. These effects of limonoids seem to be stimulant-like on the activities of the central nervous system.

In this paper, we describe the effects of seven analogous limonoids on sleeping time induced by anesthetics and the relationship between the structure and the effects of limonoids on sleeping time.

Experimental

Plant Materials Pellodendri Cortex was purchased from Uchida Wakan-yaku, the seeds of *Citrus depressa* SIEB. ex TANAKA were collected in Okinawa Prefecture in December and the seeds of *Citrus junos* HAYATA were collected in Kochi Prefecture in late October.

Chemicals Sodium borohydride ($NaBH_4$) was purchased from Kanto Chemical Co., Inc. and urethane was obtained from Sigma Chemical Co. Other reagents were purchased from Wako Pure Chemical Ind.

Animals Male ddY mice (4 weeks of age) weighing 20 to 22 g purchased from Japan SLC, Inc. (Shizuoka), and kept for one week before use.

Isolation and Modification of Limonoids **1**, **4** and nomilin (**7**) were isolated from P. Cortex, the seed of *C. depressa* and *C. junos* according to the reported methods.[4] 7α-obakunol (**2**) and 7β-obakunol (**3**) were prepared by reduction of **1** with $NaBH_4$.[5] 7α-limonol (**5**) and 7β-limonol (**6**) were also prepared by reduction of **4** with isopropyl alcohol in the presence of aluminium isopropoxide and with $NaBH_4$ respectively.[6]

Assay Samples for assays were suspended in aqueous 2% gum Arabic solution. The oral administration volume of the test sample was 0.1 ml/10 g of body weight. Each sample of limonoids was administered to the mice orally at a dose level of 200 mg/kg/d (**1**, 4.41×10^{-4}; **2** and **3**, 4.39×10^{-4}; **4**, 4.26×10^{-4}; **5** and **6**, 4.24×10^{-4}; **7**, 3.89×10^{-4} mol/kg/d) for 5 d. Aqueous 2% gum Arabic was administered to the control group. Mice were provided with foods (MF, Oriental Yeast Industry Co., Ltd.) and water *ad libitum*. For measurement of sleeping time, α-chloralose (50 mg/kg) and urethane (500 mg/kg) in physiological saline was administered intraperitoneally to 14 or more mice in each individual group.

The sleeping time was calculated as the period from the loss of righting reflex till the time of recovery of righting reflex 3 times in 1 min.

Statistical Analyses Duncan's test was used in statistical comparisons. p values of less than 0.05 were considered to be significant.

Results and Discussion

As described in the previous paper,[3] **1** and **4** isolated from P. Cortex (Rutaceae) shortened the sleeping time induced in mice by α-chloralose and urethane. Both **1** and **4** are typical limonoids in edible citrus fruits, therefore these limonoids may have some effects in humans. In addition, since these limonoids may affect sleeping time which is related to brain function, limonoids are important compounds to investigate in studying the mechanisms of hypnosis and the central nervous system in the brain. In this paper, we present the effects of **1**, **4** and other limonoids on sleeping time induced by anesthetics and the relationship between the structure and the effects of limonoids on sleeping time.

1, **4**, and **7** were isolated from the seeds of citrus fruits and P. Cortex. **2** and **3** were prepared by reduction of **1**.

5 and **6** were obtained by reduction of **4**. **1**, **2** and **3** are called the "obakunone group," a tentative group name, and **4**, **5** and **6** are called the "limonin group" in this paper.

Figure 1 shows the effects of the obakunone group on sleeping time induced in mice by α-chloralose and

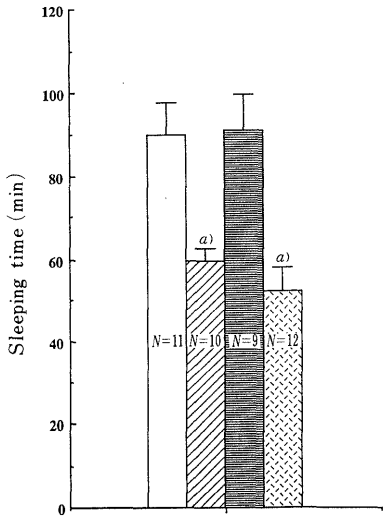

Fig. 1. Effects of Obakunone Group on Sleeping Time Induced in Mice by α-Chloralose and Urethane (50 and 500 mg/kg respectively, i.p.)

□, control; ▨, obakunone (**1**); ▤, 7α-obakunol (**2**); ▩, 7β-obakunol (**3**). Mean±S.E. *a*) $p<0.01$.

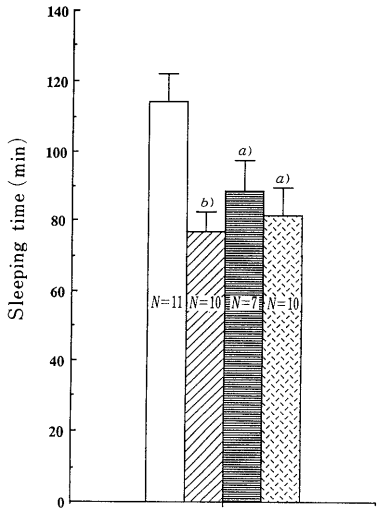

Fig. 2. Effects of Limonin Group on Sleeping Time Induced in Mice by α-Chloralose and Urethane (50 and 500 mg/kg respectively, i.p.)

□, control; ▨, limonin (**4**); ▤, 7α-limonol (**5**); ▩, 7β-limonol (**6**). Mean±S.E. *a*) $p<0.05$, *b*) $p<0.01$.

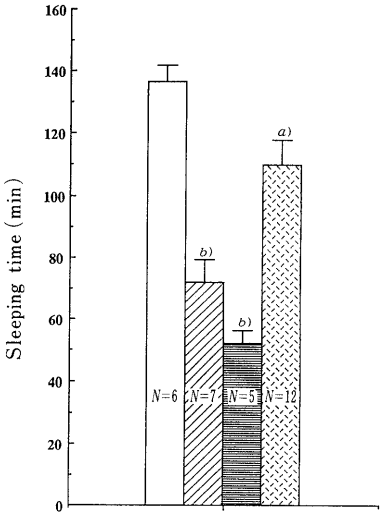

Fig. 3. Effects of Limonoids on Sleeping Time Induced in Mice by α-Chloralose and Urethane (50 and 500 mg/kg respectively, i.p.)

□, control; ▨, obakunone (**1**); ▤, nomilin (**7**); ▩, limonin (**4**). Mean±S.E. *a*) $p<0.05$, *b*) $p<0.01$.

urethane. Both **1** and **3** shortened the sleeping time compared with the control, however, there was no significant difference in the case of **2**. **1** and **3** had approximately the same reduction rates[7] of sleeping time (34% and 42%, respectively). These results suggested that the substitution groups and/or configuration of the C-7 position in the obakunone group may be related to the effects (Fig. 4).

All of the limonin group, that is **4**, **5** and **6**, shortened the sleeping time, however, their reduction rates (33%, 22% and 29%, respectively) were slightly lower than those of the obakunone group (Fig. 2). There were no significant differences among each other in the limonin group. These results suggested that the steric and substitutional factors of the C-7 position in the limonin group had little influence on the reduction rate of sleeping time.

Figure 3 shows the comparison of **1**, **4** and **7** on sleeping time. **7** had the highest reduction rate and 8 in 14 mice did not lose the righting reflex. The order of the reduction rate

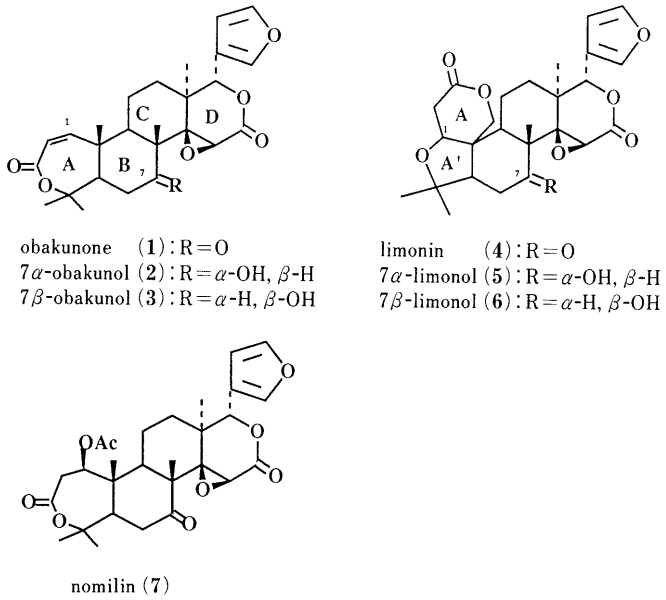

Fig. 4. Structure of Limonoids and Those Derivatives

of sleeping time was as follows; **7**>**1**>**4**. **7** is regarded as the derivative of **1**, because **7** is obtained formally by the addition of acetic acid to **1**. Therefore, the stereochemical effects of the A-ring in those limonoids may contribute to the relative strength of the reduction rate on sleeping time, because the difference of the basal skeleton between the obakunone group including **7** and the limonin group is only that of the A-ring (Fig. 4).

From the above-mentioned results, the order of the reduction rate of sleeping time was as follows; **7**>**1** and **3**>**4**, **5** and **6**. **2** had no effects on sleeping time.

In the preliminary experiments, 0.1% of **1** or **4** or **7** in powdered feed did not affect the sleeping time induced by hexobarbital.[8] The occurrence of the effects of limonoids on sleeping time seems to be related to the differences of anesthetic mechanisms between urethane and hexobarbital.

Although the mechanisms of sleeping are complex, limonoids are important compounds to investigate in studying the mechanisms of hypnosis and the central nervous system in the brain.

References and Notes

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7) Reduction rate (%) was calculated as follows;

$$\text{reduction rate} = \left(1 - \frac{\text{sleeping time of treated group}}{\text{sleeping time of control}}\right) \times 100 .$$

8) The sleeping time in the case of hexobarbital (90 mg/kg, i.p.) was as follows; control, 28.4±2.5 min; obakunone, 29.7±1.6 min; limonin, 32.2±3.5 min; nomilin, 29.5±1.9 min.
Each sample was mixed with powdered feed to constitute 0.1% limonoid (period of feeding, for 4 d). Average food intake of a mouse was *ca.* 4 g/d.